IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Ebrahim ZANDI, et al.

Title: COMPOSITION AND METHOD FOR RECONSTITUTING IkB KINASE IN YEAST AND METHODS OF USING SAME

Appl. No.: 10/079,949

Filing Date: 2/19/2002

Examiner: Prouty, Rebecca E.

Art Unit: 1652

Confirmation Number: 6542

DECLARATION UNDER 37 CFR SECTION 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. We, Ebrahim Zandi and Beth Schomer Miller, hereby declare as follows.

2. We are the Ebrahim Zandi and Beth Schomer Miller, who are named as co-inventors of the above-identified application.

3. That we conceived and reduced to practice in the United States the transformation of an IKK subunit gamma (γ) gene, an IKK subunit alpha (α) gene and/or an IKK subunit beta (β) gene into yeast and the separation from that yeast a substantially homogenous and biologically functional IKK protein complex prior to November 15, 2000, the online publication date of the literature article Li et al. (2001) "Role of IKKγ/NEMO in Assembly of the IκB Kinase Complex"

Journal of Biological Chemistry 276(6):4494-4500. Attached hereto is Exhibit A, a copy of pages from laboratory notebooks recorded by Beth Schomer Miller working under our direct control and supervision showing a reduction to practice wherein the activity of a purified IKK complex from yeast transformed with either IKKβ, IKKβγ, or IKKαβγ compared to mammalian IKK complex isolated from control Hela cells or TNF stimulated HELA cells was determined. These experimental results demonstrate that a yeast cell was transformed with an IKK subunit gamma (γ) gene, an IKK subunit alpha (α) gene and/or an IKK subunit beta (β) gene. The yeast was then grown and a substantially homogenous and biologically functional IKK protein complex was separated from the yeast.

4. That the documents in Exhibit A, which relates to the aforementioned actual reduction to practice, are exact and true copies. All personal information, including names and dates have been redacted from the documents, but all dates are prior to November 15, 2000.

5. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are true; and further that all statements made herein are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United Sates Code, and that such false statements may jeopardize the validity of the patent application currently being examined and any patent issued thereon.

Respectfully submitted,

Ebrahim Zandi

Ebrahim Zandi — Signature:

06/24/2008 — Date:

Beth Schomer Miller

_____ Signature:

_____ Date:

Atty. Dkt. No. 064189-0501

Beth Schomer Miller

Beth Schomer Miller
Signature:

June 25, 2008
Date:

Exhibit A

TOPS 36161
MADE IN U. S. A.

[redacted] Purpose: to compare IKK activity in
y αβγ yβγ yβ HNS TNF

Blue GF βγ 8.25 good (HA) signal in 2 sec exp.
SML Fr 10 or 11

Blue GF αβγ good α + γ 1min SML Fr 10-11
1λ yαβγ
HA good signal 15 sec similar to

1λ of [redacted] γ→βγ Fr 10

Blue β HA detect some Fr 15 after 1min (15λ)

HNS GF [redacted]
HNS β detected in 10 + 11 aft 1min 20λ
TNF weakly detected in 10 + 11 40min 20λ
INP. identical.

Hugo's westerns were also poor for detection of
β in β alone + αβγ in his assays.
I'll have to play around with amounts

HNS Q20 + TNF Q20 were separated by gel filtration
INP. could detect ~~III~~ 5-15λ by αIKK β + γ western
in 15 sec.

Less present than 5λ y IKK

Put fractions in gel filtration → ~10 fold dilution
would need to use 150λ for same amt.
150(10) + 150(11)
Concentrate ~~300~~λ → 30λ

use: 5, 10, 15

[redacted]

B-HA fraction 15 G.F.
3 ✓ + 12 ✓ 1x KA
5 ✓ + 10 ✓ 1x
10 ✓ + 5 ✓ 1x
[redacted]

BX -HA Fr 10
3 ✓ + 12 ✓ 1x
5 ✓ + 10 ✓ 1x
10 ✓ + 5 ✓ 1x
[redacted]

αBX Fr 10-11
3 ✓ + 12 ✓ 1x
5 ✓ + 10 ✓ 1x

HNS Q 20 → Sp 6 GF 10 & 11 200 + 200 → 40 ✓
use 5, 10, 15 ✓
10 ✓ + 5 ✓ 1x

TNF Q 20 → Spo 6 GF 10 & 11
200 ✓ + 200 ✓ → 40 ✓
use 5, 10, 15 ✓
10 ✓ + 5 ✓ 1x

Load 35 ✓ each

1	empty ✓	
2	empty ✓	
3	B3 ✓	
4	5 ✓	
5	10 ✓	
6	BX 3 ✓	
7	5 ✓	all
8	10 ✓	carefully
9	αBX 3 ✓	labeled
10	5 ✓	
11	HNS 5 ✓	
12	10 ✓	
13	5 ✓	
14	TNF 5 ✓	
15	10 ✓	
16	5 ✓	
17	MW ✓	

UltraFree MC
50. KD
put
300 ✓ 1x Kinase buffer in bottom ↓ prevent drying ↑ filter

Should recover
≤ 40 ✓ retentate
recover
~~200~~ 25 ✓ HNS
add 15 ✓ 1x
40 ✓ TNF

1. aliquot extract + buffer according to table
2. add 30 ✓ Kinase cocktail Inc 30' 30°C
3. add 4x SDS PAGE, heat
4. Load 10 ✓ gel

Only 6 ✓ each

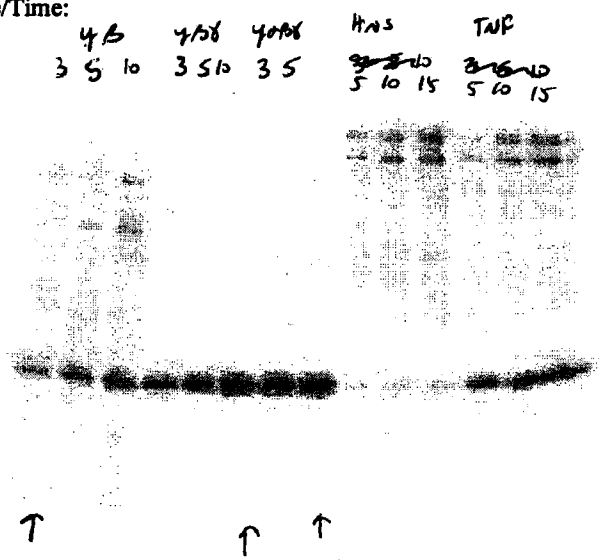
Cocktail - 15
10x Kinase 45 ✓
20mM DTT 45 ✓
200μM ATP 45 ✓
0.5 ng/μL GST-Hck 30 ✓
γ ATP 7.5 [redacted] ✓ 90Ci-5x ✓ 3 ✓ 90Ci-5x ✓
H2O [redacted] 277.5 ✓ 414.5 ✓

[redacted] Purpose: to compare activity of
yB vs yBT vs yaBT vs IHNS vs TNF

10% gel (10-10)			Stack
30% acryl		5 ml	1.05
8.8		3.75	1.9 (6.8)
H_2O		6.25	4.5
APS	2000	100	75
TEMED	200	10	10

File/Range: D:\Users\1012bsm.gel / 0.000-45853 Counts / 1.000000
User Name: phospho
Image Name: D:\Users\1012bsm.gel
Image Comment: yeast b bg abg HNS TNF-Hela
scanned 9:13 am to2:05 pm

Present Date/Time: [redacted]
Scan Date/Time:
Prep. Date/Time:



range 1-10,000

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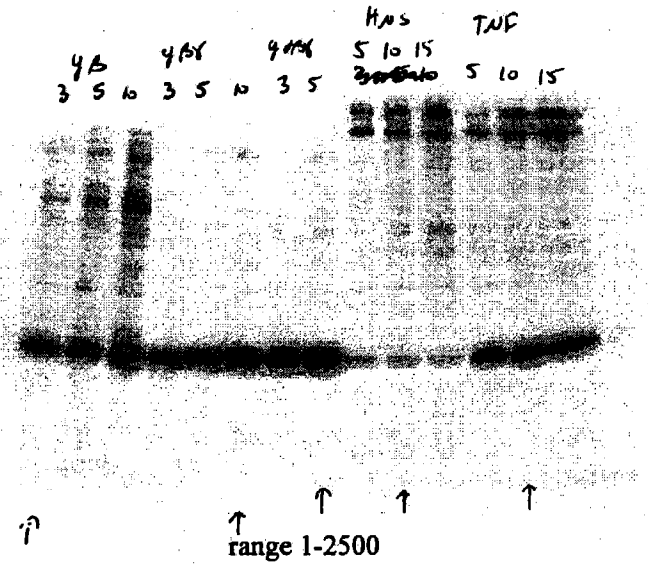
Image Name: D:\Users\1012bsm.gel
Image Comment: yeast b bg abg HNS TNF-Hela
scanned 9:13 am to2:05 pm

Present Date/Time: [redacted]
Scan Date/Time:
Prep. Date/Time:



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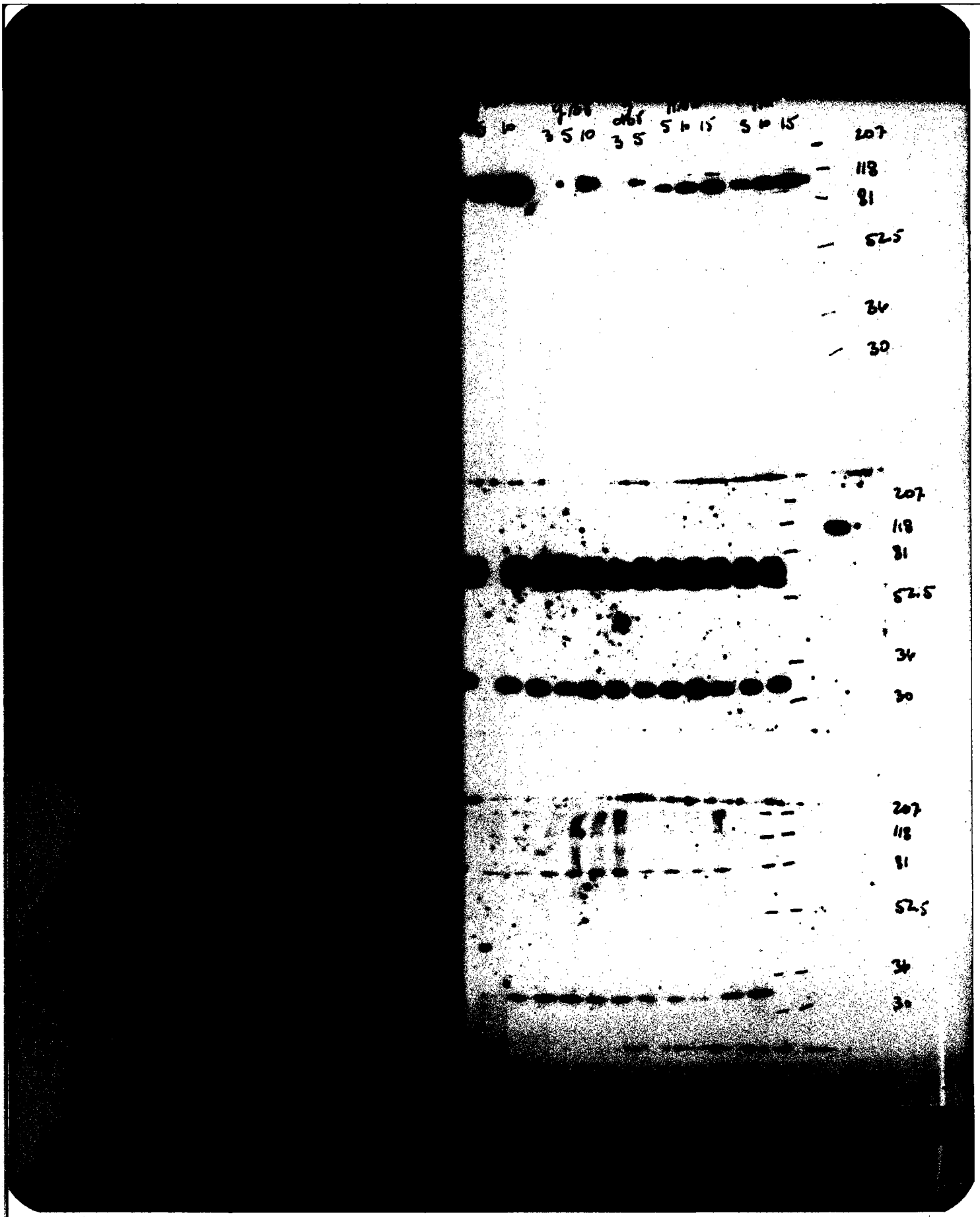
3 5 10
3 5
5 10 15
5 10 15
207
118
81
52.5
34
30
207
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81
52.5
34
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207
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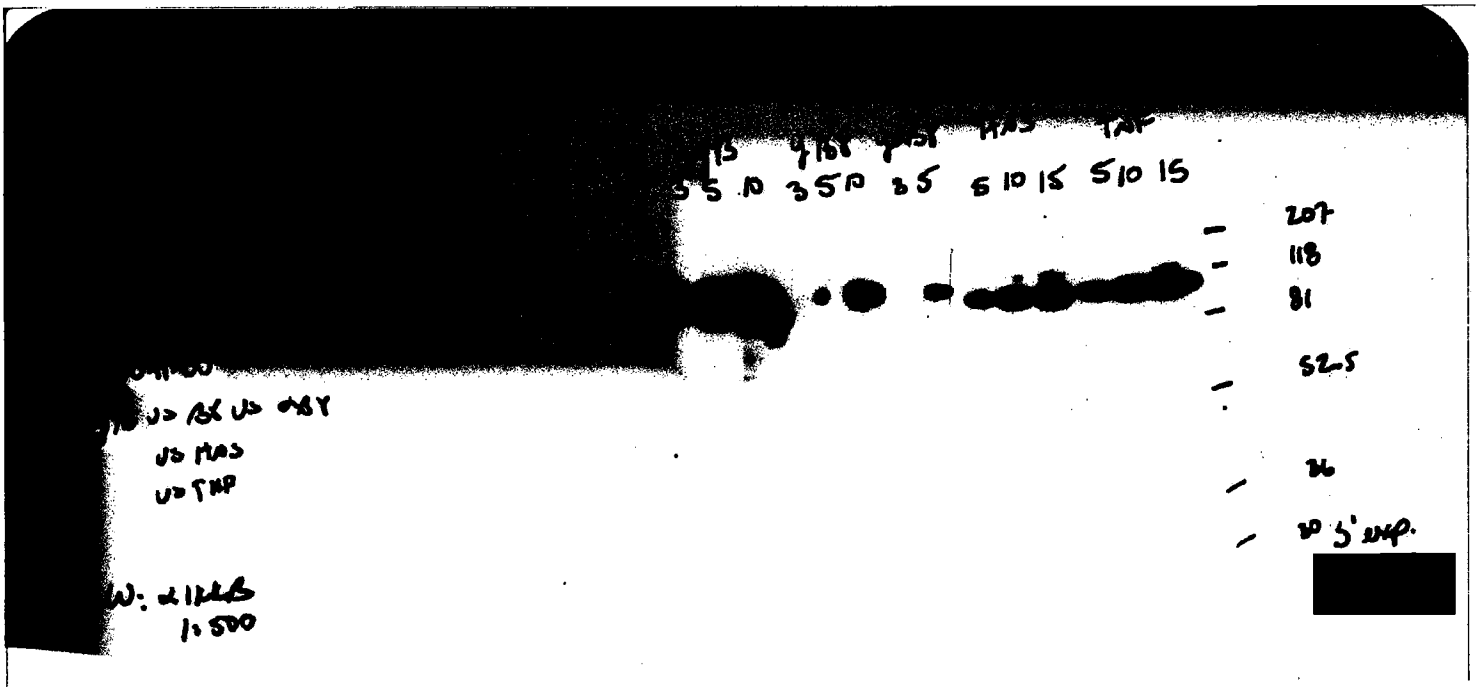
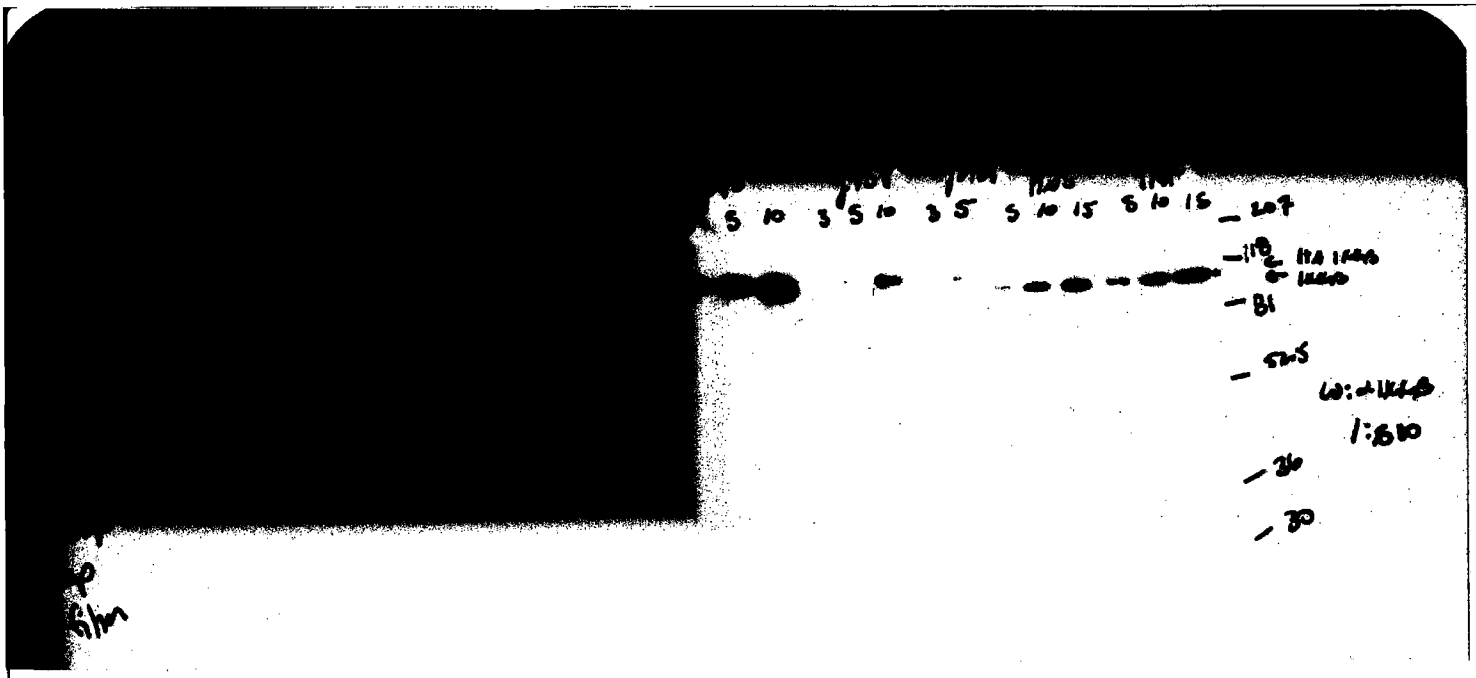
52.5
34
30


5 5 10 35 10 5 5 10 15 5 10 15
207
118
81
525
36
10 5' exp.
UD ABC UD ABY
UD MAS
UD TAP
W: 1:500


5 10 3 5 10 3 5 5 10 15 5 10 15
207
118
Bi
52.5
1:500
36
30

[redacted] Purpose: to compare IKK activity in
yΔ vs yβr vs. yαβr vs HNS vs TNF-Hela

repeat of 10-11 with attempt to use more similar amounts

HNS & TNF (Q20 → sup6 GF 10 + 11)
Put 300 µl 1x IKinase buffer in bottom to prevent drying.
Top: 200 µl sup6 GF 10 + 200 µl sup6 GF 11

Recover ~ 400 µl + adjust vol. to 400 µl
(1x IKA)

β-HA fraction 15

Tube/Lane		5 µl + 45 µl 1x ✓ Dilute 1:10 in 1x Kinase	
2	0.5 µl	5 µl	+ 16 µl 1x
3	1 µl	10 µl	+ 11 µl 1x
4	2 µl	20 µl	+ 1 µl 1x
5 βα -	7 µl	8 µl (Hela TNF)	+ 14 µl 1x
6	14 µl	~10	+ 7 µl 1x
7	21 µl	~15	+0
8 +βα	7		+ 14 µl 1x
9	14		+ 7 µl 1x
10	21		+0
11 HNS	5		+ 16 µl 1x
12	10		+ 11 µl 1x
13	15		+ 6 µl 1x
14 TNF	5		+ 16 µl 1x
15	10		+ 11 µl 1x
16	15		+ 6 µl 1x
17 mw			

21
35
56

all loaded correctly / 40 µl each.

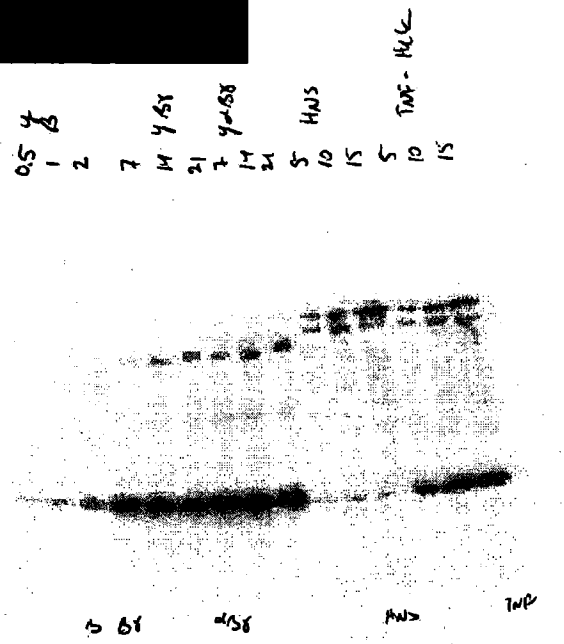
1. Aliquot extract + buffer 35 µl
2. Add 5 µl Kinase Cocktail Inc 30' 30°C
3. Add 12 µl 6x SDS PAGE Heat 95°C 5'
4. Load 10% gel (46 µl)

Cocktail	16 samples	+ 4
10x Kinase	48 µl	12 µl
20mM DTT	48 µl	12 µl
200µM ATP	48 µl	12 µl
GST-IKBα	32 µl	8 µl
32P ATP	8 µl	906-58 2 µl
H_2O	296	74
	480	120

1M DTT
20 mM DTT
.02 1M + .98 H_2O

File/Range: D:\Users\1017bsm.gel / 0.000-45853 Counts / 0.814331
User Name: phospho
Image Name: D:\Users\1017bsm.gel
Image Comment: 2 experiments
1. 3 M urea GF column fractions (concentrated)
2. yeast b, bg, abg, HNS, TNF stim Hela

Present Date/Time: [redacted]
Scan Date/Time:
Prep. Date/Time:



scale 1-2500

File/Range: D:\Users\1017bsm.gel / 0.000-45853 Counts / 0.814331
User Name: phospho
Image Name: D:\Users\1017bsm.gel
Image Comment: 2 experiments
1. 3 M urea GF column fractions (concentrated)
2. yeast b, bg, abg, HNS, TNF stim Hela

Present Date/Time: [redacted]
Scan Date/Time:
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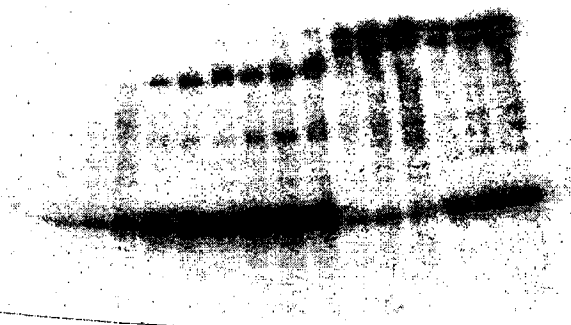

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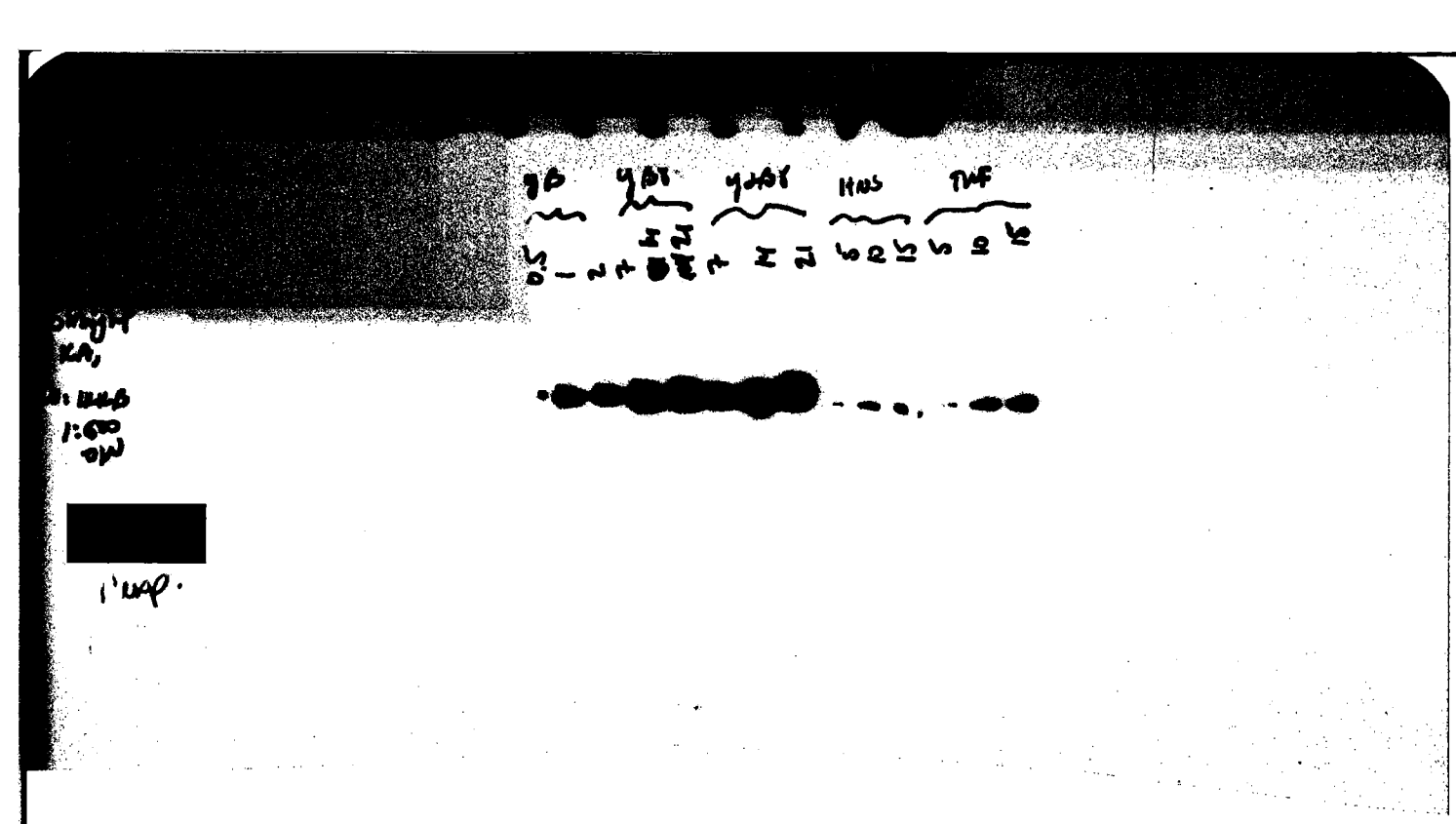

kDa
30' exp.